



RESEARCH PAPER

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Frequency of *Plasmodium vivax* and *Plasmodium falciparum* malaria in school going children of Quetta (City), Balochistan

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Abstract

Malaria is one of the major health problem caused by a vector borne disease of the genus, *plasmodium*. This parasite is transmitted through bite of female mosquito, Anopheles called malarial vector. The present work was conducted during 1st August 2017 to 31st July 2018 to examine the frequency rate of malarial infection in school going children of Quetta (city) A total no. of 2213 blood slides were prepared (thin & thick) from 33 different schools of Quetta (city) visited twice in each month out of 2213/433 are positive for the malaria infection. The overall positivity rate was (19.5%). However *P. vivax* was found to be the most common specie with the highest frequency rate (84.52%) as compared with that of *P. falciparum* (6.01%) and mixed infection was also seen in the present study (12.29%). The variations were found in different age groups. The higher frequency rate of *P.vivax* (90.8%) in the age group of 11-16 years and lowest was found (81.4%) in the age group of 3-5 years.

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Introduction

Malaria is one of the major health problem caused by a vector borne disease of the genus, *plasmodium*. This is a parasite which is transmitted naturally by the bite of female mosquito, *Anopheles* called malarial vector (Irshad *et al.*, 2013). Malaria is endemic in Pakistan. *Plasmodium vivax* was found to be the most common specie which can be transmitted by female *Anopheles* mosquito (Khattak *et al.*, 2013). All infectious diseases malaria is also one of them that has exclusive morbidity and mortality rate.

In growing nations contamination ratio is high and one of the factors in sickness spreading is poverty. Genus *Plasmodium* having four clinical importance species: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* are most dangerous to human beings and causes (90%) deaths (Ahmad *et al.*, 2014). In children of less than 10 years have a very weak immune system due to high risk of malaria estimated 87% death rate occur in every year (WHO 2011). In the city and rural areas of Pakistan malaria is still serious health threat due to lack facilities and awareness. Malaria is primarily found in the provinces of Balochistan, KPK, Sindh, and the Federally Administered Tribal Areas. Malaria transmission is considered to be unsuitable, with major *P.vivax* transmission peaking from June to september and Again in April to June, when relapses of infection in December (Kakar *et al.*, 2010; Ahmad *et al.*, 2014].

Recently, in most geographical regions malaria transmission was recorded moderate or high and management measures consequently targeted on the protection of young children and pregnant ladies. However, increased management efforts have recently reduced the amount of malaria infection transmission in several elements of geographic area (O'Meara *et al.*, 2010; Noor *et al.*, 2014).

A Recent malaria report from Pakistan, published in 2015, evidence sustained decrease in the number of cases. However, we are unable to achieve the global targets of malaria eradication from the world (Khan *et*

al., 2014). In India *Plasmodium falciparum* is the major species that cause deaths and contributes approximately one fifth of the Population (Qureshi *et al.*, 2014). Iran was most endemic area for malaria and the incidence of malaria was very high, however, the prevalence is remarkably reduced in the years due to malarial control program (Mohammadi *et al.*, 2011).

Quetta (city) is known to be endemic for the *P. Vivax* and *P. Falciparum*, major malaria causing species. It is the leading health challenge not only in the other parts of the world but also in the continent of Asia including Pakistan with special reference to Balochistan, Quetta (city). Our research is concerned to assess the frequency rate of malaria in school going children of Quetta (city), Balochistan for effective control.

Materials and methods

Study design

The present study was carried to find out the frequency rate of malaria infection among school going children of Quetta (city). The study was conducted during 1st August 2017 to 31st July 2018. The inclusive and exclusive were defined, the students have temperature, fever, chills, vomiting was including in this study.

Those students which do not have general symptoms of malaria were excluded from the current study. The data were collected from 33 different schools of Quetta (city). Blood is the only material which is routinely used for the direct detection of malarial parasites. Standard WHO recommended methodology were used during this investigation. Malaria cases are generally detected by ACD (Active case detection) this is done by making school visits and examining the blood of students with symptoms, signs or a history suggestive of malaria present or in recent past.

Collection of blood samples

A total no. of 2213 blood samples of school going children in age range between 3-5 years, 6-10 years,

11-16 years were collected from 33 different schools of Quetta (city) twice in a month.

Preparation of blood films slides

Two kinds of blood films were ready for examination: the thick and thin films on the same slides on separate slides. When clean-up with spirit and drying, the finger-tip was pricked and gently squeezed until a good drop of blood exudes. The drop of blood was touched with a clean dry slide, close to one end. School visits were created twice in each month. Both thick and thin slides we have a tendency to ready by the techniques proposed by (Paniker, 2002).

a) Preparation of thin blood film slides

A thin blood films slide was created by spreading of drop of blood equally across of unpolluted, grease free slide with the sting of spreader slide. A properly created thin film slide was consisting of an unbroken smear of one layer of red cells.

b) Preparation of thick blood film slides

A drop of blood was taken on the middle of a glass slide and unfolds with the corner of another slide to provide a square of circular patch of moderate thickness and will be specified printed matter may be simply seen through it (Paniker, 2002). The thin and thick blood films all were fixed in methanol for 2-4 minutes and allowed to face for 15 minutes to dry. Diluted Giemsa stain was applied over both thick and thin films and allowed to face for 10-15 minutes. The slides were

than washed and dried.

Identification of malaria parasites

The stained blood slides were examined below oil immersion magnifier. The film shows the presence or absence of protozoal infection parasites in 1 or 2 fields, however in thin films species of parasites is known. In falciparum protozoal infection, the presence of cell crescents creates species identification easy. Consistent with (Paniker, 2002) several tests have been Developed, but none will replace the thick and thin smear that alone can reveal parasite morphology clearly enough for correct identification still say because the 'gold standard' in protozoal infection diagnosing (Zaman and beg, 2004)

Plasmodium species identification was created following the keys equipped by (Paniker, 2002; Cheng, 1986; Sood, 1989; Chiodini, 2001). *Plasmodium vivax* and *plasmodium falciparum* (fig.1 & 2) were distinguished by the structure of Trophozoites and gametocytes. Pictures were enamored the assistance of light microscope (CH3 Olympus, japan) fitted with camera.

Results

Total no. of 2213 blood smears were prepared from the school going children of Quetta (city) in different age groups ranging from 3-5 years, 6- 10 years and 11-16 years. However variation was observed in the present study.

Table 1. School-wise frequency rate of malarial infection in school going children of Quetta(city).

S. No	Name of schools	Total no. of slides examined	Total no. of +ve Slides	<i>P.vivax</i>	<i>P.falci:</i>	Mixed infection
1	Govt High School Killi Sheikhan Quetta	96	22 (22.9%)	21 (95.4%)	01 (4.5%)	-
2	Dar-Ul-Ilm Public Middle School Muslimabad Colony Quetta	85	13 (15.2%)	11 (84.6%)	-	02 (15.3%)
3	Quetta Residential School Quetta	80	20 (25%)	16 (80%)	04 (20%)	-
4	Pak Unity Model School Satellite-Town Quetta	75	13 (17.3%)	10 (76.9%)	-	03 (23%)
5	Shees Public Model High School Resani Road Quetta	86	10 (11.6%)	08 (80%)	02 (20%)	-

6	Rehmat Public High School Sariab Road, Quetta	95	24 (25.2%)	22 (91.6%)	02 (8.3%)	-
7	Bolan Public Model School Sabzal Road Quetta	50	05 (11%)	04 (80%)	-	01 (20%)
8	Babar English Medium High School Quetta	90	10 (11.1)	07 (70%)	03 (30%)	-
9	Govt Girls High School Killi Kamalo.	83	22 (26.5%)	19 (86.3%)	-	03 (13.6%)
10	Govt Boys High School Wahdat Colony Quetta	78	13 (16.6%)	12 (92.3%)	01 (7.6%)	-
11	Brilliant Future Public High School Quetta	98	20 (20.4%)	18 (90%)	-	02 (10%)
12	Govt: Primary School Killi Ragma Quetta	56	06 (10.7%)	03 (50%)	03 (50%)	-
13	The Citizen Foundation Boys Campus Secondary School Quetta.	69	14 (20.2%)	12 (84.7%)	-	02 (14.2%)
14	The Nation Secondary School Girls Campus Quetta.	67	13 (19.4%)	09 (69.2%)	04 (30%)	-
15	Govt Girls Middle School Killi Kasi Abad, Quetta.	76	16 (21%)	14 (87.5%)	-	02 (12.5%)
16	Good Way Public Model School (R) Bank Colony Quetta	40	23 (57.5%)	21 (91.3%)	-	02 (8.6 %)
17	Govt Girls High School ChashmaKhuddoDeba Quetta	98	16 (16.3%)	14 (87.5%)	-	02 (12.5%)
18	Bright Kids Public High School Quetta	76	23 (30.2%)	20 (86.9%)	-	03 (13.0%)
19	Govt Girls Middle School Shamozaï Quetta.	56	11 (19.6%)	10 (90.9%)	01 (9.0%)	-
20	IqraRozat-Ul-Quran Lil Atfal Trust Killi Shakhan Quetta	67	14 (20.8%)	12 (85.7%)	-	02 (14.2%)
21	The Foundation School Quetta	78	07 (8.9%)	05 (71.4%)	-	02 (28.5%)
22	Govt Primary School Killi Sheikhan Quetta	89	23 (25.8%)	20 (86.9%)	-	03 (13.0%)
23	Iqra Residential School And College Quetta	65	10 (15.3%)	09 (90%)	01 (10%)	-
24	The Gate Of Knowledge School System Quetta	56	18 (32.1%)	16 (88.8%)	-	02 (11.1%)
25	J.G.S (Girls Section) Naw Killi Quetta	76	08 (10.5%)	07 (87.5%)	-	01 (12.5%)
26	Cambridge Secondary School Nawakilli, Branch Quetta	45	06 (13.3%)	05 (83.3%)	-	01 (16.6%)
27	Laraib Public High School Quetta.	62	10 (22.2%)	06 (60%)	-	04 (40%)
28	Pak Saudia Grammar School System Quetta	39	08 (20.5%)	07 (87.5%)	-	01 (12.5%)
29	Skillful Grammar Public School Quetta	19	03 (15.7%)	02 (66.6%)	-	01 (33.3%)
30	South City School System Quetta	23	07 (30.4)	05 (71.4%)	-	02 (28.5%)

31	Times Schooling System Quetta	34	03 (8.8%)	02 (66.6%)	-	01 (33.3%)
32	British High School Sariab Road Quetta	56	12 (21.4%)	10 (83.3%)	-	02 (16.6%)
33	Govt: Girls high school police line Quetta	50	10 (20%)	09 (90%)	-	01 (10%)
Total		2213	433 (19.5%)	366 (84.5%)	22 (6.01%)	45 (12.2%)

In (table I), the over-all frequency of Plasmodium slides positivity rate was (2213/433) (19.56%) where is *P.vivax* (fig.1) was found with highest rate(83.52%) followed by *P. falciparum* (fig.2) (6.01%) and mixed infection was seen (12.29%). *P.vivax* was found to be

more common and observed with the highest frequency of (95.4%) in Govt: high school killi sheikhan Quetta and lowest rate in Govt: Primary school killi ragha Quetta.

Table 2. Age-wise overall frequency rate of malaria infection in school going children of Quetta(city).

S. No.	Age group in years	Total no. of slides examined	Total No. of (+ ve) slides	Overall % Infection	Inf. By <i>P.vivax</i>	Inf. by <i>P.falciparum</i>	Mixed infection
1	3-5	852	172	20.18%	140	6	26
2	6-10	759	141	18.57%	117	10	14
3	11-16	602	120	19.93%	109	06	05
Total		2213	433	19.56%	366	22	45

In (table II) the variations in different age groups were also seen the overall frequency of positivity rate was (19.56%) with the higher infection of *P. vivax* (84.5%) than *P. falciparum* (6.01%) and mixed infection (12.29%). Highest infection was observed (90.8%) in the age group of 11-16 and low frequency rate was (81.4%) seen in the age group of 3-5years. In (table III) the data were statistically analyzed whether there is any association between the age groups and types of malarial infection.

The data were statistically analyzed to test whether there is any Association between different type of infection and age groups through a chi-square-test formula mentioned by (Neil, WA.1982.).

$$x^2 = \text{cal} \sum \frac{(fo - fe)^2}{fe} = 10.985$$

The chi-square value of 10.985 (p-value= 0.027) was calculated. A significant association was found between these two attributes (*Plasmodium vivax* and *Plasmodium falciparum*) at 5% level of significance.

The occurrence of malaria infection *P. vivax* (type A) was found larger than other types.

Discussion

In the present study, total no. of 2213 blood smears were prepared from different schools of Quetta (city) in different age group ranging from 3-5 years 6-10 years and 11-16 years in 33 different schools.

The overall slides positively rate was (19.56 %) where *P. vivax* was higher (84.52%) than *P. falciparum* (6.01%) and mixed infection (12.29%). School wise frequency was observed highest of *P. vivax* (95.4%) in Govt High School Killi Sheikhan Quetta and lowest (50%) in Govt: Primary School Killi Ragha Quetta.

Age wise frequency was observed highest (20.18%) in the age group of 3-5 years. Where *P. vivax* (81.4%) *P. falciparum* (3.5%) and mixed infection (15.1%).

The same study on malaria reported *P. vivax*(2.4%) from Punjab province and Sindh (10.8%), in Islamabad (3.8%) and in Balochistan *P.vivax* (76%), *P. falciparum* (18%) and (6%) were mixed infection (Khattak *et al.*, 2013).

According to malaria control program Balochistan (MCPB) *P.vivax* was observed to be higher (14.2%) in 2016 and (21.3%) in 2017. (MCPB 2016-2017).

Another research work reported from school going male children among age range between 4 -5 years were more affected (2.5%) than 1-2 years children (2.4%). Prevalence of *P.vivax* (6.33%) was found to be highest in pre-school going boys youngsters followed

by *P. falciparum* (0.23%) and mixed infection (0.12%). Highest share (2.73%) of affected pre-school going female youngsters was within the age range between 4 to 5 years. Whereas lowest percentage (1.44%) was discovered within the age vary between 2 to 3 years. Among pre-school going female youngsters, highest share (7.11%) was affected with *P.vivax* whereas *P. falciparum* and mixed infection wasn't discovered (Zeb *et al.*, 2015).

Table 3. Statistical analysis.

S.NO	Age group	Types of infection						Total
		A		B		C		
		(fo)	(fe)	(fo)	(fe)	(fo)	(fe)	
1	3-5	140	145.3	06	8.73	26	17.87	172
2	6-10	117	119.1	10	7.16	14	14.65	141
3	11-16	109	101.4	06	6.09	5	12.47	120
Total		366		22		45		433

As the new report indicates the prevalence of malaria parasites infection including both infections has decreased significantly across sub Saharan Africa since 2000, in sub Saharan Africa Average infection

prevalence rate in children aged 2-10 years fell from 26% in 2000 to 14% in 2013, a relative decline of 46% (WHO 2014).

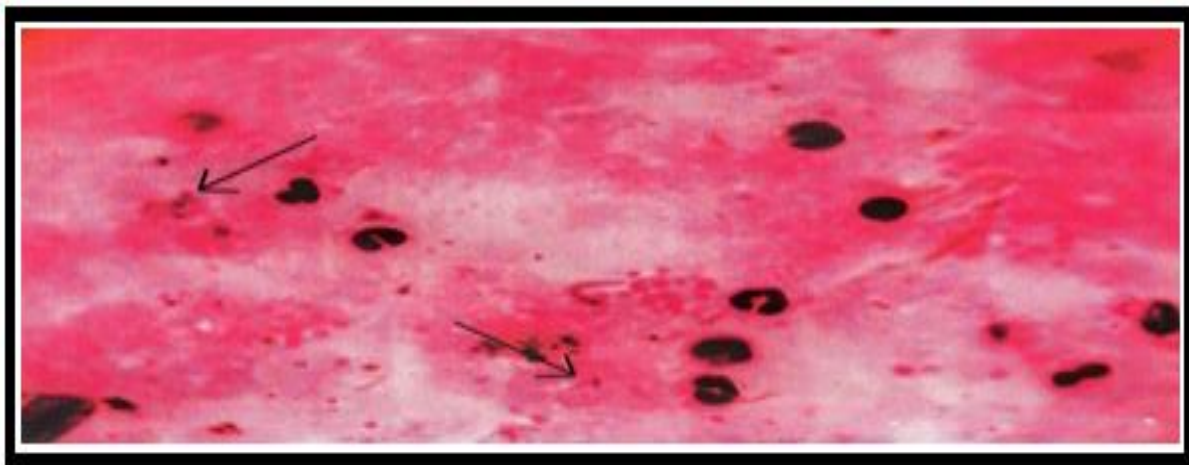


Fig. 1. Showing Gametocytes of *P.vivax* thick blood smear (100x).

The study was conducted in bordering area of Balochistan province with Sistan are reported 247 cases of malaria in 7 year old children. They also studied the prevalence of malarial species in Sarbaz, Iran and reported that malaria is more common among boys and in rural residents. They further reported that most cases occurred during autumn and the lowest in winter. *Plasmodium Vivax* was the most

common type of Plasmodium species (Shuja *et al.*, 2016). Similar to other studies in Sistan and Baluchistan province malaria was more common in boys.

Probably its cause was the amount of body covered by clothing, which in Iranian boys is less than girls. Also, compared to girls, boys are more likely to be present

at night outside the home. Therefore, boys due to of higher exposure to vectors, have higher risk of malaria. At current study, approximately 70.9% of patients lived in rural areas. Similarly in the study

conducted by Youssefi and Rahimi in Sarbaz County, that 91% of patients were lived in rural areas (Khattak *et al.*, 2013; WHO 2014).

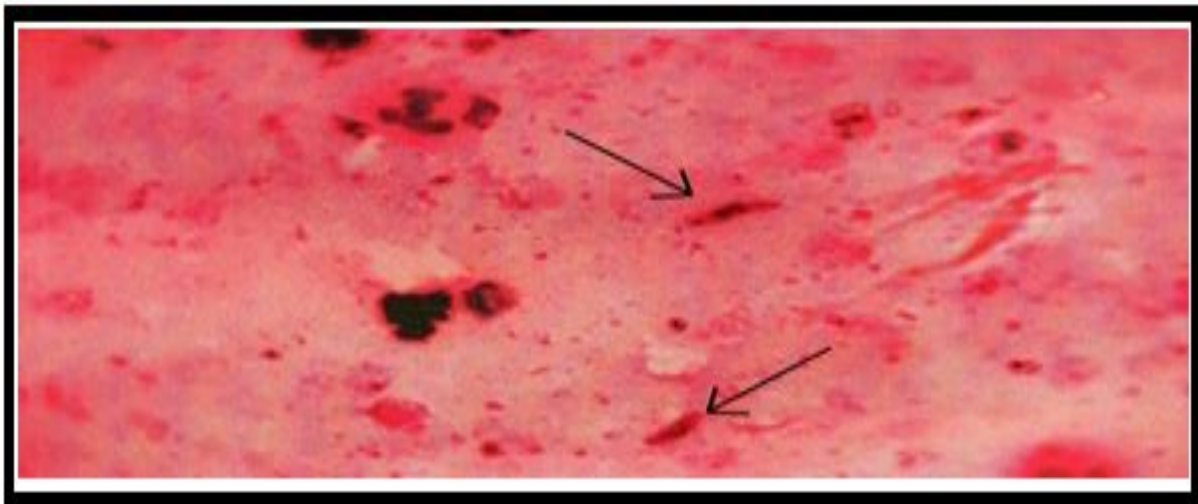


Fig. 2. Showing gametocytes of *P. falciparum* thick blood smear (100x).

These results suggest that the risk of malaria is higher in rural areas as compared to city. (Reza *et al.*, 2011).

Conclusion

In conclusion *P. vivax*, *P. falciparum* and mixed infection were observed in school going children using Microscopy.

The frequency of *P. vivax* was higher as compared to *P. falciparum* infection both genders (males/females) in city areas. We strongly recommended malarial symptomatic school going children with the current study areas should be properly treated with antimalarial drugs to avoid life loss.

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